

## Quantitation of biological speciation using hyphenated AMS

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Accelerator MS is a form of isotope-ratio, tandem MS in which a “dissociation cell” is held at megaVolt potentials. An ion beam is accelerated to MeV energies after an initial keV mass analysis at single AMU resolution. The high velocity of the ions causes complete destruction of molecular isobars in the form of hydrides. Further magnetic and electric sector filtering is used before each ion is uniquely identified through nuclear counting methods to distinguish any nuclear isobars.

(AMS) was developed twenty years ago to serve as a radiocarbon dating tool in geochemistry, archaeology, and oceanography. AMS is more than a million times more efficient than decay counting in detecting radiocarbon. Samples containing attomoles (femtoCuries) of radiocarbon are routinely quantified to better than 1% precision and accuracy for these applications. We began eight years ago the development of sample handling methods to use AMS sensitivity for biological and environmental tracing using molecules labeled with radiocarbon at low specific activities. Similar efficiencies of detection are achieved to carbon dating, but at lower precision due to the preparation processes needed to keep sample crosstalk and contamination to a minimum. Absolute quantitation is easily obtained. We use AMS to quantify chemical species defined and separated by HPLC, TLC, PAGE, CE, and simple physical dissection in studies of pharmacokinetics, metabolism, and in macromolecular binding.

The limit of quantitation (LOQ) of our AMS system is approximately 10 attomoles determined through serial dilutions and by statistical analysis of HPLC blanks. Direct AMS quantitation can be made over a range of 10<sup>4</sup>. Precision and

accuracy are better than 5% over much of the range. The extreme care required to reduce accidental contamination of a sample at ppq sensitivity has reduced expected “animal” variations to less than a few percent. Since only the radiocarbon is quantified and preservation of structure is not important, samples maintain stable integrity after biochemical definition. We are able to “rescue” archived samples from experiments in which decay counting is unable to quantify the isotopic label. The correlation to scintillation is 99.99% or greater using sample sizes 500 or more times smaller than those used in scintillation counting.

We show a number applications of hyphenated AMS with the quantified results.

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